

c) monitoring activation of said GPCR by complementation of the first and second mutant forms of the reporter enzyme;

wherein increased reporter enzyme activity in the cell compared to that which occurs in the absence of the test condition indicates increased GPCR interaction with the modified arrestin compared to that which occurs in the absence of the test condition, and decreased reporter enzyme activity in the cell compared to that which occurs in the absence of the test condition indicates decreased GPCR interaction with the modified arrestin compared to that which occurs in the absence of the test condition.

2. (Amended) A method of assessing the effect of a test condition on G-protein-coupled receptor (GPCR) pathway activity, comprising:

B<sup>1</sup> a) providing a cell that expresses a GPCR as a fusion protein to a first mutant form of reporter enzyme and an interacting protein partner as a fusion to a second mutant form of the reporter enzyme;

wherein the GPCR fusion protein is modified to include one or more sets of serine/threonine clusters, wherein said one or more sets of serine/threonine clusters enhance binding of said GPCR to arrestin, wherein said enhanced binding between said GPCR and said arrestin increases sensitivity of detection of said effect of said test condition;

b) exposing the cell to a ligand for the GPCR under said test condition; and

c) monitoring activation of the GPCR by complementation of said reporter enzyme;

wherein increased reporter enzyme activity in the cell compared to that which occurs in the absence of the test condition indicates increased GPCR interaction with the interacting protein partner compared to that which occurs in the absence of the test condition, and decreased reporter enzyme activity in the cell compared to that which occurs in the absence of the test

condition indicates decreased GPCR interaction with interacting protein partner compared to that which occurs in the absence of the test condition.

3. (Amended) A DNA molecule comprising a sequence encoding a biologically active hybrid GPCR, wherein the hybrid GPCR comprises a GPCR as a fusion protein to a mutant form of reporter enzyme and wherein the hybrid GPCR is modified to include one or more sets of serine/threonine clusters, wherein the one or more sets of serine/threonine clusters enhance binding of said hybrid GPCR to an arrestin.

4. (Amended) A DNA construct capable of directing the expression of a biologically active hybrid GPCR in a cell, comprising the following operatively linked elements:

a promoter; and

B<sup>1</sup> a DNA molecule comprising a sequence encoding a biologically active hybrid GPCR, wherein the hybrid GPCR comprises a GPCR as a fusion protein to one mutant form of reporter enzyme and wherein the hybrid GPCR is modified to include one or more sets of serine/threonine clusters, wherein the one or more sets of serine/threonine clusters enhance binding of the hybrid GPCR to an arrestin.

5. (Amended) A cell transformed with a DNA construct capable of expressing a biologically active hybrid GPCR in a cell, comprising the following operatively linked elements:

a promoter; and

a DNA molecule comprising a sequence encoding a biologically active hybrid GPCR, wherein the hybrid GPCR comprises a GPCR as a fusion protein to one mutant form of reporter enzyme and wherein the hybrid GPCR is modified to include one or more sets of serine/threonine clusters, wherein the one or more sets of serine/threonine clusters enhance binding of the hybrid GPCR to an arrestin.

6. (Twice Amended) A DNA molecule comprising a sequence encoding an arrestin as a fusion protein to a mutant form of a reporter enzyme, wherein the arrestin is modified to enhance binding of the arrestin to a GPCR.

7. (Twice Amended) A DNA construct comprising the following operatively linked elements:

a promoter; and

a DNA molecule comprising a sequence encoding an arrestin as a fusion protein to a mutant form of a reporter enzyme, wherein the arrestin is modified to enhance binding of said arrestin to a GPCR.

8. (Twice Amended) A cell transformed with a DNA construct comprising the following operatively linked elements:

a promoter; and

a DNA molecule comprising a sequence encoding an arrestin as a fusion protein to a mutant form of a reporter enzyme, wherein the arrestin is modified to enhance binding of the arrestin to a GPCR.

9. (Twice Amended) A method of assessing the effect of a test condition on G-protein-coupled receptor (GPCR) pathway activity, comprising:

a) providing a cell that expresses a GPCR as a fusion protein to a first mutant form of a reporter enzyme and an arrestin as a fusion protein to a second mutant form of the reporter enzyme complementary to the first mutant form of the reporter enzyme,

wherein the arrestin is modified by introducing a point mutation in a phosphorylation-recognition domain to remove a requirement for phosphorylation of the GPCR for arrestin binding to permit binding of the arrestin to said GPCR in the cell regardless of whether the GPCR is phosphorylated,

- b) exposing the cell to a ligand for said GPCR under the test condition; and
- c) monitoring activation of the GPCR by complementation of the first and second mutant forms of the reporter enzyme;

wherein increased reporter enzyme activity in the cell compared to that which occurs in the absence of the test condition indicates increased GPCR interaction with the modified arrestin compared to that which occurs in the absence of the test condition, and decreased reporter enzyme activity in the cell compared to that which occurs in the absence of the test condition indicates decreased GPCR interaction with the modified arrestin compared to that which occurs in the absence of the test condition.

10. (Amended) The method of Claim 9, wherein the arrestin is mutated to increase a property selected from affinity and avidity for activated, non-phosphorylated GPCR.

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11. (Amended) The method of Claim 10, wherein the arrestin is  $\beta$ -arrestin2 and wherein the  $\beta$ -arrestin2 is mutated to convert Arg169 to an oppositely charged residue.

12. (Amended) The method of Claim 11, wherein the oppositely charged residue is selected from the group consisting of histidine, tyrosine, phenylalanine and threonine.

13. (Amended) The method of Claim 9, wherein the arrestin is mutated to increase a property selected from affinity and avidity for activated and phosphorylated GPCR.

14. (Amended) A method of assessing the effect of a test condition on G-protein-coupled receptor (GPCR) pathway activity, comprising:

- a) providing a cell that expresses a GPCR as a fusion protein to a first mutant form of a reporter enzyme and an interacting protein partner as a fusion to a second mutant form of the reporter enzyme;

wherein the GPCR fusion protein is modified to include one or more serine/threonine clusters, the one or more serine/threonine clusters defined as serine or threonine residues

occupying three consecutive or three out of four consecutive positions, wherein said one or more serine/threonine clusters enhance binding of the GPCR to arrestin, wherein the enhanced binding between the GPCR and the arrestin increases sensitivity of detection of the effect of the test condition;

b) exposing the cell to a ligand for the GPCR under the test condition; and

c) monitoring activation of the GPCR by complementation of the reporter enzyme;

wherein increased reporter enzyme activity in the cell compared to that which occurs in the absence of the test condition indicates increased GPCR interaction with the interacting protein partner compared to that which occurs in the absence of the test condition, and decreased reporter enzyme activity in the cell compared to that which occurs in the absence of the test condition indicates decreased GPCR interaction with interacting protein partner compared to that which occurs in the absence of the test condition.

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15. (Amended) The method of Claim 1, wherein the modified arrestin exhibits enhanced binding to activated, phosphorylated GPCR.

16. (Twice Amended) The method of Claim 1, wherein the modified arrestin comprises conversion of Arg169 to an amino acid selected from the group consisting of histidine, tyrosine, phenylalanine and threonine.

17. (Twice Amended) The method of Claim 1, wherein the modified arrestin comprises conversion of Val170 to alanine.

18. (Twice Amended) The method of Claim 1, wherein the arrestin is selected from the group consisting of  $\beta$ -arrestin1 and  $\beta$ -arrestin2, and wherein the  $\beta$ -arrestin1 or the  $\beta$ -arrestin2 is truncated for all or part of a carboxyl-terminal half of the  $\beta$ -arrestin1 or the  $\beta$ -arrestin2.

19. (Amended) The method of Claim 18, wherein the  $\beta$ -arrestin1 or the  $\beta$ -arrestin2 is truncated from amino acid 190 of the  $\beta$ -arrestin1 or the  $\beta$ -arrestin2 to the carboxyl-terminal end of the  $\beta$ -arrestin1 or the  $\beta$ -arrestin2.

20. (Twice Amended) The method of Claim 1, wherein the arrestin is a chimera of  $\beta$ -arrestin1,  $\beta$ -arrestin2 and/or visual arrestin.

21. (Amended) The method of Claim 10, wherein the arrestin is a chimera of  $\beta$ -arrestin1,  $\beta$ -arrestin2 and/or visual arrestin.

22. (Amended) The method of Claim 11, wherein the arrestin is a chimera of  $\beta$ -arrestin1,  $\beta$ -arrestin2 and/or visual arrestin.

B<sup>1</sup> 23. (Amended) The method of Claim 12, wherein the arrestin is a chimera of  $\beta$ -arrestin1,  $\beta$ -arrestin2 and/or visual arrestin.

24. (Twice Amended) The method of Claim 10, wherein the arrestin is  $\beta$ -arrestin2 and wherein the  $\beta$ -arrestin2 is mutated to convert Arg170 to an oppositely charged residue.

25. (Twice Amended) The method of Claim 1, wherein the modified arrestin comprises conversion of Arg170 to an amino acid selected from the group consisting of histidine, tyrosine, phenylalanine and threonine.

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Please add the following new claims:

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-- 26. (New) The method of Claim 1, wherein the GPCR and the first mutant form of reporter enzyme are linked together by a polypeptide linker represented by the formula - (GGGGS)<sub>n</sub>-.

B<sup>2</sup> 27. (New) The method of Claim 26, wherein n is 2 or more.

28. (New) The method of Claim 26, wherein n is 4.

29. (New) The method of Claim 1, wherein the second mutant form of the reporter enzyme is linked to the C-terminal of the arrestin.

30. (New) The method of Claim 2, wherein the modified GPCR fusion protein comprises a full length GPCR linked to the first mutant form of reporter enzyme by a first polypeptide linker comprising one or more serine/threonine clusters.

31. (New) The method of Claim 2, wherein the protein partner is an arrestin.

32. (New) The method of Claim 31, wherein the first mutant form of reporter enzyme is linked to the first polypeptide linker by a second polypeptide linker represented by the formula - (GGGGS)<sub>n</sub>-.

33. (New) The method of Claim 32, wherein n is 2 or more.

34. (New) The method of Claim 32, wherein n is 4.

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35. (New) The method of Claim 31, wherein the second mutant form of the reporter enzyme is linked to the C-terminal of the arrestin.

36. (New) The DNA molecule of Claim 3, wherein the hybrid GPCR comprises a full length GPCR linked to the mutant form of reporter enzyme by a first polypeptide linker comprising one or more serine/threonine clusters.

37. (New) The DNA molecule of Claim 36, wherein the mutant form of reporter enzyme is linked to the first polypeptide linker by a second polypeptide linker represented by the formula - (GGGGS)<sub>n</sub>-.

38. (New) The DNA molecule of Claim 37, wherein n is 2 or more.

39. (New) The DNA molecule of Claim 37, wherein n is 4.

40. (New) The DNA construct of Claim 4, wherein the hybrid GPCR comprises a full length GPCR linked to the mutant form of reporter enzyme by a first polypeptide linker comprising one or more serine/threonine clusters.

41. (New) The DNA construct of Claim 40, wherein the mutant form of reporter enzyme is linked to the first polypeptide linker by a second polypeptide linker represented by the formula  $-(GGGGS)_n-$ .

42. (New) The DNA construct of Claim 41, wherein n is 2 or more.

43. (New) The DNA construct of Claim 41, wherein n is 4.

44. (New) The cell of Claim 5, wherein the hybrid GPCR comprises a full length GPCR linked to the mutant form of reporter enzyme by a first polypeptide linker comprising one or more serine/threonine clusters.

45. (New) The cell of Claim 44, wherein the mutant form of reporter enzyme is linked to the first polypeptide linker by a second polypeptide linker represented by the formula -  $(GGGGS)_n-$ .

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46. (New) The cell of Claim 45, wherein n is 2 or more.

47. (New) The cell of Claim 45, wherein n is 4.

48. (New) The DNA molecule of Claim 6, wherein the mutant form of the reporter enzyme is linked to the C-terminal of the arrestin.

49. (New) The DNA construct of Claim 7, wherein the mutant form of the reporter enzyme is linked to the C-terminal of the arrestin.

50. (New) The cell of Claim 8, wherein the mutant form of the reporter enzyme is linked to the C-terminal of the arrestin.

51. (New) The method of Claim 9, wherein the GPCR and the first mutant form of reporter enzyme are linked together by a polypeptide linker represented by the formula -  $(GGGGS)_n-$ .

52. (New) The method of Claim 51, wherein n is 2 or more.

53. (New) The method of Claim 51, wherein n is 4.